SHORT COMMUNICATION

Inefficient Mechanical Transmission of Langat (Tick-Borne Encephalitis Virus Complex) Virus by Blood-Feeding Mites (Acari) to Laboratory Mice

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J. Med. Entomol. 30(3): 639–641 (1993)

ABSTRACT One day after feeding on a viremic mouse, tropical rat mites, *Ornithonyssus bacoti* (Hirst), transmitted Langat (tick-borne encephalitis virus complex) virus to a naive suckling mouse in one of four trials. However, no transmissions to naive mice by *O. bacoti* were recorded either immediately after the viremic blood meal (0/4 trials) or on days 4–18 (0/20 trials). After feeding on a viremic mouse, chicken mites, *Dermanyssus gallinae* (De Geer), failed to transmit Langat virus to naive suckling mice in any trials (0/24). Although virus failed to replicate in either species of mite, it was detectable in 20% (2/10) of *O. bacoti* individuals 1 d after a viremic blood meal, but only immediately after the viremic blood meal in 20% (2/10) of *D. gallinae* mites. Neither mite appears to be an efficient vector of Langat virus.

KEY WORDS Langat virus, vector competency, mites

LANGAT (LGT) VIRUS is a member of the tick-borne encephalitis (TBE) virus complex (genus *Flavivirus*, family Flaviviridae). Infections in healthy persons may cause fever, but in leukemic patients this virus can produce encephalitis (Webb et al. 1966, Begum 1969). LGT virus has been isolated from three species of ixodid ticks: *Haemaphysalis papuana* Thorell in Thailand, *Ixodes granulatus* Supino in Malaysia, and *Ixodes persulcatus* Schulze in central Siberia (Smith 1956, Bancroft et al. 1976, Karabatsos 1985). This virus has also been isolated from Malaysian forest rats, which may serve as reservoir hosts (Smith 1956, Karabatsos 1985). Transmission of LGT virus by tick bite has been demonstrated experimentally for *Ixodes ricinus* (L.) and *Haemaphysalis spinigera* Neumann, and replication and transstadial transmission of the virus has been demonstrated in *Dermacentor marginatus* (Sulzer) (Varma & Smith 1962, Karabatsos 1985).

No isolations of LGT virus have been reported from blood-feeding mites. However, the antigenically similar TBE (tick-borne encephalitis) virus has been isolated from hematophagous mesostigmatid mites in nature and has also been shown to persist in experimentally infected mites for up to 63 d (Naumov & Gutova 1984, Kocianova & Kozuch 1988). Therefore, the potential for two species of widely distributed hematophagous mesostigmatid mites to transmit LGT virus from viremic to naive suckling mice was investigated under laboratory conditions.

Separate, virus-free laboratory colonies of the tropical rat mite, *Ornithonyssus bacoti* (Hirst), and the chicken mite, *Dermanyssus gallinae* (De Geer), were maintained as described previously (Durden & Linthicum 1992, Durden et al. 1992). One-day-old suckling mouse (Harlan-Sprague ICR strain) littermates were inoculated intraperitoneally with $10^{3.3}$ plaque-forming units (PFU) of the TP 21 strain of LGT virus. This strain, obtained from the Yale Arbovirus Research Unit, was originally isolated from *I. granulatus* collected in the Ulu Langat Forest reserve, Malaysia, in 1956 (Smith 1956) and had been passaged eight times in suckling mice and three times in Vero cells before use in these experiments. Three days after inoculation, eight of the LGT-inoculated mice were removed and each was placed into a separate mite-proof container. Sixty unfed, uninfected, female *O. bacoti* were allowed to feed on each of four of these

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care. The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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mice, while 60 unfed, uninfected, female *D. gallinae* were allowed to feed on each of the remaining four mice. Mites were allowed to feed for 2 h, at which time each mouse was anesthetized with CO_2 and blood (0.1 ml) was collected by cardiac puncture. Blood was mixed 1:10 in diluent (10% fetal bovine serum in Medium 199 with Hanks' salts and antibiotics) and frozen at −70°C. Immediately after mites had fed on the viremic mouse and on days 1, 4, 8, 12, and 18, 8–10 of the virus-exposed mites of each species were allowed to feed for 2 h on a naive suckling mouse (one mouse per trial for each species of mite). These suckling mice were then returned to the dam and monitored daily over the next 21 d for signs of viral infection. Moribund mice were killed with CO_2, blood and brain samples were collected, and tissue suspensions were prepared by mixing 1:10 in diluent and then frozen at −70°C. After 21 d, surviving test mice were anesthetized with CO_2 and 0.5–1 ml of blood was collected by cardiac puncture. These blood samples were allowed to clot at room temperature and sera were removed and tested for specific IgG antibody to LGT virus by using an enzyme-linked immunosorbent assay (ELISA). Immediately after they had fed on a viremic mouse or on days 1, 4, 8, 12, and 18, individual mites were triturated in 1 ml of diluent and frozen at −70°C. Blood, brain, and mite samples were thawed at a later date, clarified by centrifugation at 1,000 × *g* for 10 min, and tested for virus by plaque assay (Earley et al. 1967) on confluent monolayers of 4- to 5-d-old LLC-MK_2 cells (a rhesus monkey kidney cell line). Blood and brain samples of moribund mice were also tested by ELISA for LGT virus.

Viremia levels in the eight virus-inoculated suckling mice immediately after mite feeding were $10^{7.1}$ to $10^{7.5}$ PFU per ml of blood. Virus was transmitted by *O. bacoti* to a naive suckling mouse in one of four trials 1 d after the mites had fed on a viremic suckling mouse. No other transmissions were detected (0/20 for *O. bacoti*, 0/24 for *D. gallinae*). The single transmission by *O. bacoti* was evidenced by a moribund suckling mouse 4 d after virus-exposed mites had fed and by positive plaque-assay ($10^{7.9}$ PFU per ml of brain tissue) and ELISA results. One additional suckling mouse that was fed upon by virus-exposed *O. bacoti* became moribund, but brain and blood samples were negative for virus (cause of morbidity unknown). All 21-d sera from surviving mice were negative for specific IgG antibody to LGT virus by ELISA.

Virus was detected in 50% (5/10) (mean positive titer = $10^{1.6}$ PFU per mite) of the *O. bacoti* sampled immediately after they had fed on a viremic mouse. This percentage dropped to 20% (2/10, mean positive titer = $10^{1.2}$ PFU per mite) after 1 d, and to 0% (0/40) after 4–18 d. Comparable data for *D. gallinae* were 20% (2/10, mean positive titer = $10^{1.2}$ PFU per mite) immediately after the viremic blood meal and 0% (0/50) after 1–18 d.

Neither *O. bacoti* nor *D. gallinae* was an efficient vector of LGT virus in our transmission trials. The failure of LGT virus to replicate in either species of mite indicates that the single transmission by *O. bacoti* 1 d after the viremic meal was probably mechanical rather than biological. These data suggest that blood-feeding mites are probably not involved in the natural transmission cycle of LGT virus.

Acknowledgments

We thank J. H. Oliver, Jr. (Georgia Southern University, Statesboro, GA) for providing chicken mites for colony establishment, R. Shope (Yale Arbovirus Research Unit, New Haven, CT) and L. Iacono-Connors (USAMRIID, Fort Detrick, Frederick, MD) for providing the stock of Langat virus used, and C. Rossi (USAMRIID) for performing ELISA tests. L.A.D. was supported by a Senior Research Fellowship awarded by the National Research Council, Washington, DC.

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Received for publication 14 September 1992; accepted 25 November 1992.